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Two newly recorded invasive alien ascidians (Chordata, Tunicata, Ascidiacea) based on morphological and molecular phylogenetic analysis in Korea*

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Abstract

We report five alien ascidians with some distinct features that were investigated from August 2009 to October 2011 in Korea, among which *Ascidiella aspersa* (Müller, 1776) belonging to family Ascidiidae of order Phlebobranchia and *Molgula manhattensis* (De Kay, 1843) belonging to family Molgulidae of order Stolidobranchia are newly discovered invasive species. These ascidians were identified and their phylogenetic relationships were clarified through molecular analysis using about 680 bp of nuclear 18S rDNA and about 670 bp of mt-COI genes along with detailed morphological characteristics, and reported for the first time in Korea. It was discovered that *A. aspersa* was widespread three coastlines of Korea except Jeju Island, and *M. manhattensis* first found in Mokpo, Gunsan, and Incheon in June 2010 extended into Busan of Korea Strait in 2011.

Key words: Ascidians, *Ascidiella aspersa*, *Molgula manhattensis*, invasive alien species, Korea, morphological characters, 18S rDNA, mt-COI, molecular phylogenetic analysis

Introduction

Ascidians consist of the largest and most diverse class Ascidiacea belonging to subphylum Tunicata of phylum Chordata (Shenkar *et al*. 2012). They comprise approximately 3,000 described species found in all marine habitats from shallow water to the deep sea (Shenkar & Swalla 2011). All ascidians—commonly called sea squirts—are sessile organisms that live attached to submerged natural and artificial surfaces including rocks, pilings, ropes, and shells or carapaces of other species (Inglis *et al*. 2008). They exhibit large variations from small inconspicuous colonial forms to large and colorful solitary forms (Petersen 2007), and feed by pumping water into the body through an oral siphon. Food particles are filtered out of the water inside the body, and then expelled through an atrial siphon (Inglis *et al*. 2008). The transport of species on the hulls and in the ballast water of international shipping and the subsequent establishment of organisms in foreign ports are not new phenomena (Byrne *et al*. 1997). Ascidians are one of the key ecological groups because of their invasive potentials and abilities to thrive in eutrophic environments. For example, the solitary ascidians, *Styela clava* and *Ciona intestinalis*, have caused an adverse effect on aquaculture along Canada's east coast, mainly on mussel culture (Shenkar & Swalla 2011). Introductions of non-indigenous ascidians into harbors in both tropical and temperate waters are now commonplace, with the rate of introductions increasing, sometimes creating severe damage to natural fauna by overgrowth (Lambert & Lambert 1998; Coles *et al*. 1999; Lambert 2002; Cohen *et al*. 2005). The invasive ascidians can survive to compete with the native indigenous filter feeding organisms for food and space (Currie *et al*. 1998). They also cause huge problems for the growers in terms of costs and labors in relation to extra handling of the equipment, but potentially also with decreased bivalve production owing to the competition for foods (Petersen 2007).

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Despite their key positions in the tree of life, our comprehension of the phylogenetic affinities within the tunicates is still limited. Traditionally, tunicates have been classified into three major classes such as Ascidiacea, Appendicularia and Thaliacea with distinct life-history traits and developmental modes (Tsagkogeorga *et al*. 2009). The class Ascidiacea has commonly been classified on the basis of the structure of their branchial sac into three orders: Aplousobranchia, Phlebobranchia and Stolidobranchia (Lahille 1886). This is the current classification used by most ascidian taxonomists and also corresponds to the molecular phylogenetic analysis based on the nuclear 18S rDNA (Zeng & Swalla 2005; Tsagkogeorga *et al*. 2009) as opposed to Perrier (1898)'s division that was based upon the position of the gonads and other morphological considerations and comprised only two orders: Enterogona and Pleurogona. Ascidians belonging to the order Aplousobranchia are all colonial while the Phlebobranchia and Stolidobranchia include both colonial and solitary species (Zeng & Swalla 2005).

Molecular data have been applied to the study of phylogeny of subphylum Tunicata (Wada 1998; Swalla *et al*. 2000; Zeng & Swalla 2005; Delsuc *et al*. 2006), providing a useful tool to analyze the phylogenetic relationships within the class Ascidiacea (Wada *et al*. 1992; Stach & Turbeville 2002; Zeng *et al*. 2006). Within the Tunicata, a conserved 1 kb portion of the 18S rDNA resolves the relationships between ascidian families much better than mitochondrial gene (Hadfield *et al*. 1995; Wada 1998; Swalla *et al*. 2000; Stach & Turbeville 2002; Zeng *et al*. 2006). The mitochondrial cytochrome *c* oxidase subunit (mt-COI) gene with its high variability, has been the molecule of choice in studies of population genetics and phylogeography (Avise 2000), and has been used in ascidians to address cryptic speciation and invasions (Turon & López-Legentil 2004). In this study, partial sequences of the 18S rDNA and mt-COI of the invasive alien ascidians found in Korea have been analyzed to determine their identifications and phylogenetic relationships.

Materials and methods

Sample collection and identification. The ascidians were collected from 26 harbors or ports in Korea: 11 harbors of East Sea, 11 of Korea Strait, one of Jeju Island, and three of Yellow Sea (Fig. 1). The specimens were preserved in 95 % ethyl alcohol and were photographed alive using digital cameras (Olympus TG-310 of Olympus Co., and Canon 120IS of Canon Co., Tokyo, Japan) because the outer shape is important for identification. Some specimens were stained with aceto-carmine (*Ascidiella aspersa*, Figs. 4G, H) and methylene blue (*Molgula manhattensis*, Figs. 5G–J).

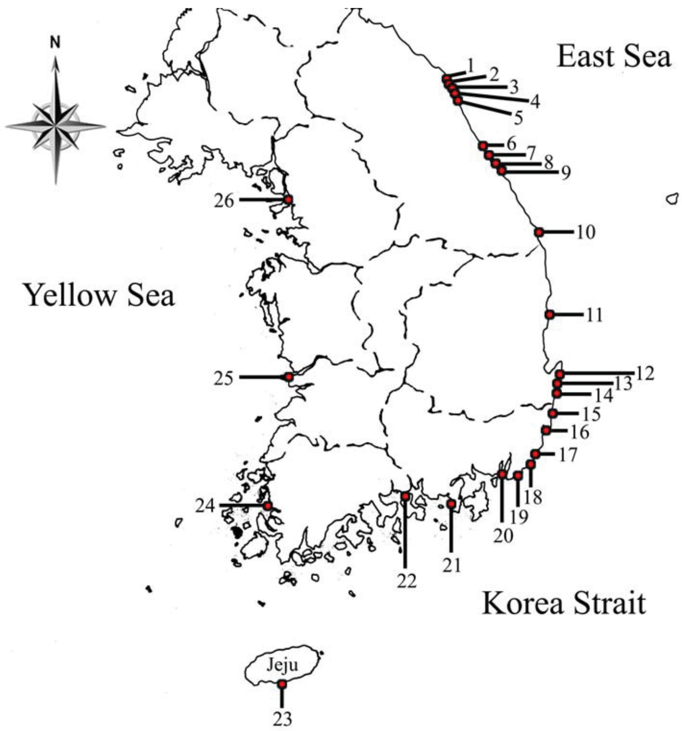

FIGURE 1. A map showing the collection sites in Korea.

DNA extraction, amplification and sequencing. Total genomic DNA was extracted from gonad tissue using the DNeasy® Tissue Kit (Qiagen). DNA fragments were amplified by polymerase chain reaction (PCR) using previously published primers (Table 1).

TABLE 1. Primers used for the amplification of 18S rDNA and mt-COI sequences.

Gene	Primer name	Primer sequence (5'–3')	Reference
18S rDNA	18S-TF	AAACGGCTACCACATCCAAG	Carreras-Carbonell *et al*. (2005)
	18S-TR	AACTAAGAACGGCCATGCAC	
mt-COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer *et al*. (1994)
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	

TABLE 2. List of ascidians examined for nuclear 18S rDNA and mt-COI genes.

Order	Family	Species	18S rDNA	mt-COI
Phlebobranchia	Ascidiidae	*Ascidia ceratodes* (Huntsman, 1912)	L12378	-
		A. sydneiensis Stimpson, 1855	AF165819	-
		A. zara Oka, 1935	AF165820	-
		Ascidiella aspersa (Müller, 1776)	*JN573230 – JN573233	*JQ742948, *JQ742949
			-	AY116600
		Ascidiella sp.	FM244843	-
	Cionidae	*Ciona intestinalis* (Linnaeus, 1767)	AB013017	-
Stolodobranchia	Molgulidae	*Molgula arenata* Stimpson, 1852	AY903919	-
		M. bleizi (Lacaze-Duthiers, 1877)	L12418	-
		M. citrina Alder and Hancock, 1848	L12420	-
		M. complanata Alder and Hancock, 1870	L12422	-
		M. manhattensis (De Kay, 1843)	*JN573245 – JN573260	*JQ742950 – JQ742955
			HM574386	HM574345
			HM574388	HM574346
		M. occidentalis Traustedt, 1883	FM244850	AY116608
		M. occulta Kupffer, 1875	L12430, L12432	-
		M. pacifica (Huntsman, 1912)	AY040738	-
		M. provisionalis Van Name, 1945	L12434	HM574376
		M. pugetiensis Herdman, 1898	AY903920	-
		M. retortiformis Verrill, 1871	AY903921	-
		M. socialis Alder, 1863	L12436	HM574379
		M. tectiformis Nishikawa, 1991	L12438	-
	Pyuridae	*Halocynthia roretzi* (Drasche, 1884)	This study	-
	Styelidae	*Styela clava* Herdman, 1881	*JN573261	*JQ742956
		S. plicata (Lesueur, 1823)	*JN573263	*JQ742957
Outgroup	Branchiostomidae	*Branchiostoma floridae* Hubbs, 1922	M97571	AF035164

*Each accession number of NCBI was obtained from our results.

PCRs were performed in a 25 µl total reaction volume with 1.0 µl of each primer (10 µM), 1.0 µl dNTPs (10 µM), 2.5 µl 10 × buffer containing $MgCl_2$ (Enzynomics: www.enzynomics.com), 0.3 µl nTaq DNA polymerase (Enzynomics) and 1.5 µl template DNA and 17.7 µl distilled water. A single soak at 94 ℃ for 2 min was followed by 35 cycles (denaturation at 94 ℃ for 45 s, annealing at specific temperatures for 50 s, and extension at 72 ℃ for 55 s) and a final extension at 72 ℃ for 7 min on a peqSTAR 96 Universal Gradient (Peqlab: www.peqlab.de). Annealing temperature was different for each gene to obtain adequate amplification (42–44 ℃ for mt-COI and 52 ℃ for 18S rDNA). Amplified products were purified with a QIAquick® PCR purification Kit (Qiagen). Purified products were sequenced on an ABI 3730XL sequencer (Applied Biosystems: www.appliedbiosystems.com) using the ABI Prism® Bigdye™ Terminator v3.1 (Applied Biosystems).

Molecular phylogenetic analysis. In order to confirm the species identities and to clarify the inter-specific relationships, we sequenced about 680 bp of nuclear 18S rDNA and about 670 bp of mt-COI for Korean ascidian specimens. Nuclear 18S rDNA and mt-COI sequences were checked and aligned using BioEdit ver. 7.0.5 (Hall 1999) and ClustalX (Thompson *et al*. 1997). The jModeltest ver. 0.1.1 (Posada 2008) was used to choose the best-fit model of nuclear 18S rDNA and mt-COI data sets with AIC criterion (GTR+I+G was calculated for both). Phylogenetic trees were inferred from Bayesian inference (BI) using MrBayes ver. 3.1.2 (Ronquist & Huelsenbeck 2003) and *Branchiostoma floridae* of subphylum Cephalochordata was chosen as an outgroup (Table 2).

Posterior probabilities were obtained by a Markov chain Monte Carlo (MCMC) algorithm with two independent runs of one cold and three heated chains. Samples of trees and parameters were drawn every 1,000 steps from a total of 4×10^6 MCMC generations. The first 1,000 trees were discarded as the burnin (based on convergence of likelihood values), and the remaining trees were used to compute a consensus tree.

Results

Subphylum Tunicata Lamarck, 1816

Class Ascidiacea Nielsen, 1995

Order Aplousobranchia Lahille, 1886

Family Clavelinidae Forbes and Hanley, 1848

Genus *Clavelina* Savigny, 1816

1. *Clavelina lepadiformis* (Müller, 1776)
(Fig. 2A, Table 3)

Ascidia lepadiformis Müller, 1776: 119, fig. 5; Sanamyan, 2010: 252057.
Clavelina lepadiformis: Milne-Edwards, 1841: 44; Alder & Hancock, 1905: 32; 1907: 152, pls. 49, 50, figs. 1, 2; Wirtz, 1998: 200; De Caralt *et al*., 2002: 125; Turon *et al*., 2003: 29; Shirley, 2003: 14, fig. 2; Riley, 2008: 3009; Reinhardt *et al*., 2010: 185, fig. 1; Sanamyan & Monniot, 2011a: 103552; Pyo & Shin, 2011: 197, fig. 1.
Claveline lepadiforme Milne-Edwards, 1841: 93.

Materials examined. 21 zooids, Daebyeon, 14 Nov. 2009, Shin S, dock wall at 2.5 m depth; 32 zooids, Busan, 14 Nov. 2009, Shin S, SCUBA diving at 20 m sea depth, S. Shin; 202 zooids, Gampo, 24 June 2010, Lee T, ropes at 2 m depth; 124 zooids, Bangeojin, 25 June 2010, Shin S, bivalve (Mytilus galloprovincialis) shells and dock wall at 1.5 m depth; 75 zooids, Seogwipo, 28 June 2010, Lee T, ropes at 5 m depth; 37 zooids, Namae, 18 Oct. 2010, Pyo J, ropes at 3.2 m depth; 44 zooids, Gampo, 19 Oct. 2010, Pyo J, dock wall at 3 m depth; 35 zooids, Bangeojin, 20 Oct. 2010, Shin S, dock wall at 2 m depth; 89 zooids, Daebyeon, 20 Oct. 2010, Shin S, fish trap at 2.3 m depth; 37 zooids., Seogwipo, 17 Oct. 2010, Pyo J, ropes at 2.4 m depth; 239 zooids, Gonghyeonjin, 22 June 2011, Lee T, dock wall at 2.5 m depth; 494 zooids, Guryoungpo, 24 June 2011, Pyo J, dock wall at 1.7 m depth; 34 zooids,

Gampo, 24 June 2011, Lee T, fish trap at 3.3 m depth; 46 zooids, Bangeojin, 25 June 2011, Shin S, under floating dock at 0.2 m depth; 79 zooids., Seogwipo, 17 June 2011, Pyo J, ropes at 3 m depth; 367 zooids, Gonghyeonjin, 16 Oct. 2011, Lee T, ropes at 3.4 m depth; 68 zooids, Guryoungpo, 18 Oct. 2011, Pyo J, dock wall at 1.5 m depth; 54 zooids, Daebyeon, 19 Oct. 2011, Shin S, fish trap at 2.1 m depth; 124 zooids., Seogwipo, 25 Oct. 2011, Pyo J, ropes at 3.6 m depth.

Distribution. Native to Norway. Northeast Atlantic (Ireland, Scotland, England, Channel Islands, France, Sweden, Portugal, Spain), Mediterranean Sea, Connecticut of Northwest Atlantic, South Africa, Japan, and Korea (Korea Strait, Jeju Island).

FIGURE 2. Five invasive alien ascidians at four collection sites in Korea: A, *C. lepadiformis* attached to dock wall at 4.7 m depth in Busan port; B, *A. aspersa* attached to ropes at Tongyeong yacht marina; C, *C. intestinalis* attached on thick cloth at Gampo harbor; D, *M. manhattensis* attached on floating buoy at Mokpo yacht marina; E, *S. plicata* attached to rope at Tongyeong yacht marina. Scale bars: A–E. 10 cm.

Order Phlebobranchia Lahille, 1886

Family Ascidiidae Herdman, 1882

Genus *Ascidiella* Roule, 1884

2. *Ascidiella aspersa* (Müller, 1776)
(Figs. 2B, 3C–D, 4, Table 3)

Ascidia aspersa Müller, 1776: 72, fig. 2.
Ascidiella aspersa: Berrill, 1928: 170; Millar, 1952: 43; Currie *et al*, 1998: 24; Stachowicz *et al*., 2002: 15497; Picton & Morrow, 2010a: ZD1410; Sanamyan & Monniot, 2011b: 103718; NIMPIS, 2012: 6000005711.

TABLE 3. The collection sites and dates of five invasive alien ascidians in Korea.

Location \ date	2009		2010		2011	
	August	November	June	October	June	October
1. Daejin				●▼	●▼	●▼
2. Chodo						●
3. Geojin						●▼
4. Gonghyeonjin					■●	■●
5. Ayajin				●▼	●	●
6. Namae				■●		●
7. Jumunjin						●▼
8. Sacheon				▼		●▼
9. Gangneung						●▼
10. Imwon						●
11. Ganggu						▼
12. Guryoungpo	▼▲	▼▲	▼	▼	■▼	■●▼
13. Yangpo			▼▲	▼▲	●▼▲	●▼▲
14. Gampo	▼▲	▼▲	▼■	■▼▲	■●▼	●▼
15. Bangeojin			■▼▲	■●▼	■●▼▲	●▼
16. Jangsaengpo	▲	▲	▼▲	●▼▲	●▲	●▼▲
17. Daebyeon	▼▲	■▼▲	▼	■●▼	●▼	■●▼
18. Songjeong						▼◆
19. Busan	▼▲	■▼▲	▼▲	▼▲	●▼▲	●▼◆▲
20. Dadaepo	▼▲	▼▲	▼▲	▼▲	▼▲	▼◆▲
21. Tongyeong	▼▲	▼▲	▼▲	●▼▲	●▼▲	●▼▲
22. Gwangyang	▼▲	▼▲	▼▲	▲	●▼◆	●▼▲
23. Seogwipo	▲	▲	■▲	■▲	■▲	■▲
24. Mokpo			▼◆	◆	●◆	●◆
25. Gunsan			▼◆	●◆	●	●
26. Incheon				◆	●◆	●◆

■ : *C. lepadiformis*, ● : *A. aspersa*, ▼ : *C. intestinalis*, ◆ : *M. manhattensis*, ▲ : *S. plicata*.

Materials examined. 12 individuals., Daejin, 17 Oct. 2010, Shin S, ropes at 2.7 m depth; 9 inds., Ayajin, 17 Oct. 2010, Pyo J, ropes at 2.7 m depth; 5 inds., Namae, 18 Oct. 2010, Shin S, dock wall at 1.7 m depth; 15 inds., Bangeojin, 20 Oct. 2010, Shin S, ropes at 2.7 m depth; 4 inds., Jangsaengpo, 20 Oct. 2010, Lee T, dock wall at 2 m depth; 8 inds., Daebyeon, 20 Oct. 2010, Pyo J, dock wall at 3 m depth; 19 inds., Tongyeong, 21 Oct. 2010, Pyo J, fish trap at 3.3 m depth; 17 inds., Gunsan, 22 Oct. 2010, Lee T, fish trap at 2.5 m depth; 27 inds., Daejin, 22 June 2011, Lee T, ropes at 1.3 m depth; 13 inds., Gonghyeonjin, 22 June 2011, Pyo J, ropes at 2.7 m depth; 7 inds.,

Ayajin, 22 June 2011, Pyo J, thick cloth at 1.5 m depth; 43 inds., Yangpo, 24 June 2011, Shin S, ropes at 2.1 m depth; 16 inds., Gampo, 24 June 2011, Shin S, thick cloth at 1.2 m depth; 9 inds., Bangeojin, 25 June 2011, Shin S, ropes at 2.7 m depth; 8 inds., Jangsaengpo, 25 June 2011, Lee T, fish trap at 2.3 m depth; 52 inds., Daebyeon, 25 June 2011, Pyo J, dock wall at 3 m depth; 8 inds., Busan, 25 June 2011, Pyo J, under floating dock at 0.2 m depth; 424 inds., Tongyeong, 26 June 2011, Pyo J, dock wall at 2.5 m depth; 21 inds., Gwangyang, 26 June 2011, Shin S, fish trap at 3.3 m depth; 12 inds., Mokpo, 26 June 2011, Lee T, fish trap at 2.5 m depth; 19 inds., Gunsan, 27 June 2011, Shin S, under floating dock at 0.3 m depth; 22 inds., Incheon, 27 June 2011, Lee T, ropes at 1.8 m depth; 23 inds., Daejin, 16 Oct. 2011, Lee T, ropes at 1.3 m depth; 5 inds., Chodo, 16 Oct. 2011, Shin S, dock wall at 1.5 m depth; 3 inds., Geojin, 16 Oct. 2011, Shin S, ropes at 1.8 m depth; 19 inds., Gonghyeonjin, 16 Oct. 2011, Pyo J, ropes at 2.7 m depth; 7 inds., Ayajin, 16 Oct. 2011, Lee T, thick cloth at 1.5 m depth; 8 inds., Namae, 17 Oct. 2011, Shin S, ropes at 2.1 m depth; 14 inds., Jumunjin, 17 Oct. 2011, Lee T, under floating dock at 0.1 m depth; 10 inds., Sacheon, 17 Oct. 2011, Pyo J, ropes at 2 m depth; 13 inds., Gangneung, 17 Oct. 2011, Shin S, thick cloth at 2.1 m depth; 6 inds., Imwon, 18 Oct. 2011, Lee T, dock wall at 1 m depth; 13 inds., Guryoungpo, 18 Oct. 2011, Pyo J, dock wall at 3 m depth; 39 inds., Yangpo, 18 Oct. 2011, Shin S, ropes at 2.1 m depth; 19 inds., Gampo, 18 Oct. 2011, Shin S, thick cloth at 1.2 m depth; 5 inds., Bangeojin, 19 Oct. 2011, Pyo J, ropes at 2.7 m depth; 11 inds., Jangsaengpo, 19 Oct. 2011, Lee T, fish trap at 3 m depth; 37 inds., Daebyeon, 19 Oct. 2011, Pyo J, dock wall at 1.1 m depth; 8 inds., Busan, 19 Oct. 2011, Pyo J, under floating dock at 0.2 m depth; 74 inds., Tongyeong, 20 Oct. 2011, Shin S, dock wall at 2.1 m depth; 15 inds., Gwangyang, 20 Oct. 2011, Shin S, fish trap at 3.2 m depth; 8 inds., Mokpo, 20 Oct. 2011, Lee T, fish trap at 2.9 m depth; 12 inds., Gunsan, 21 Oct. 2011, Shin S, under floating dock at 0.2 m depth; 24 inds., Incheon, 21 Oct. 2011, Shin S, ropes at 2.8 m depth.

FIGURE 3. Different invasive ascidians attached to same rope at different dates at Tongyeong yacht marina: A, B, *S. plicata* (Oct. 2010); C, D, *A. aspersa* (June 2011). The red arrow indicates that an individual of *S. plicata* was surrounded by several individuals of *A. aspersa*.

Description. Body heart- or irregular oval-shaped, sometimes covered with debris and fibrils. Tunic firm, thick and rough, grayish color, semi-transparent, and with numerous tiny papillae spread all over surfaces (Fig. 4L); visceral organs observed by naked eye on left side. Some pigments of reddish or heavy orange appeared near inside both siphons (Fig. 4D). Oral siphon 6–8 lobed, positioned at top of body (Fig. 4G). 19–25 slender tentacles located inner rounded orifice and well developed (Fig. 4I). Long tentacle and short one irregular arranged and longer ones outnumber shorter ones. Atrial siphon 4–6 lobed, positioned around one third of way down body away from oral siphon (Fig. 4H). Both siphons almost frilled, like flute in water. Dorsal tubercle U-shaped and with horns incurved (Fig. 4I). Branchial sac with numerous rows of straight stigmata not folded, located on right side. Body muscles usually form irregular network at mantle covering branchial sac, like string or thread (Fig. 4D). Numerous muscles of both siphons well developed. Esophagus positioned right bottom beneath end of pharynx on right side (Fig. 4F). Stomach very weak and easily damaged, short, with a longitudinal opaque white line. Intestine has first and second gut loops, located behind branchial sac. Intestinal tract from right bottom on left side, and made first gut loop upwards of half-way of body. Curved intestinal tract runs to bottom and makes second gut loop (Fig. 4E). Anus formed at end of extended gut (Fig. 4K). Gonads enclosed in first gut loop. Ovary almost fills in first gut loop. Testis surrounds ovary (Fig. 4E). Gonoduct at center of first gut loop and extends along close to outside second gut loop, opening with anus near base of atrial siphon (Fig. 4K). Numerous eggs crowded at gonads, and scattered all along gut.

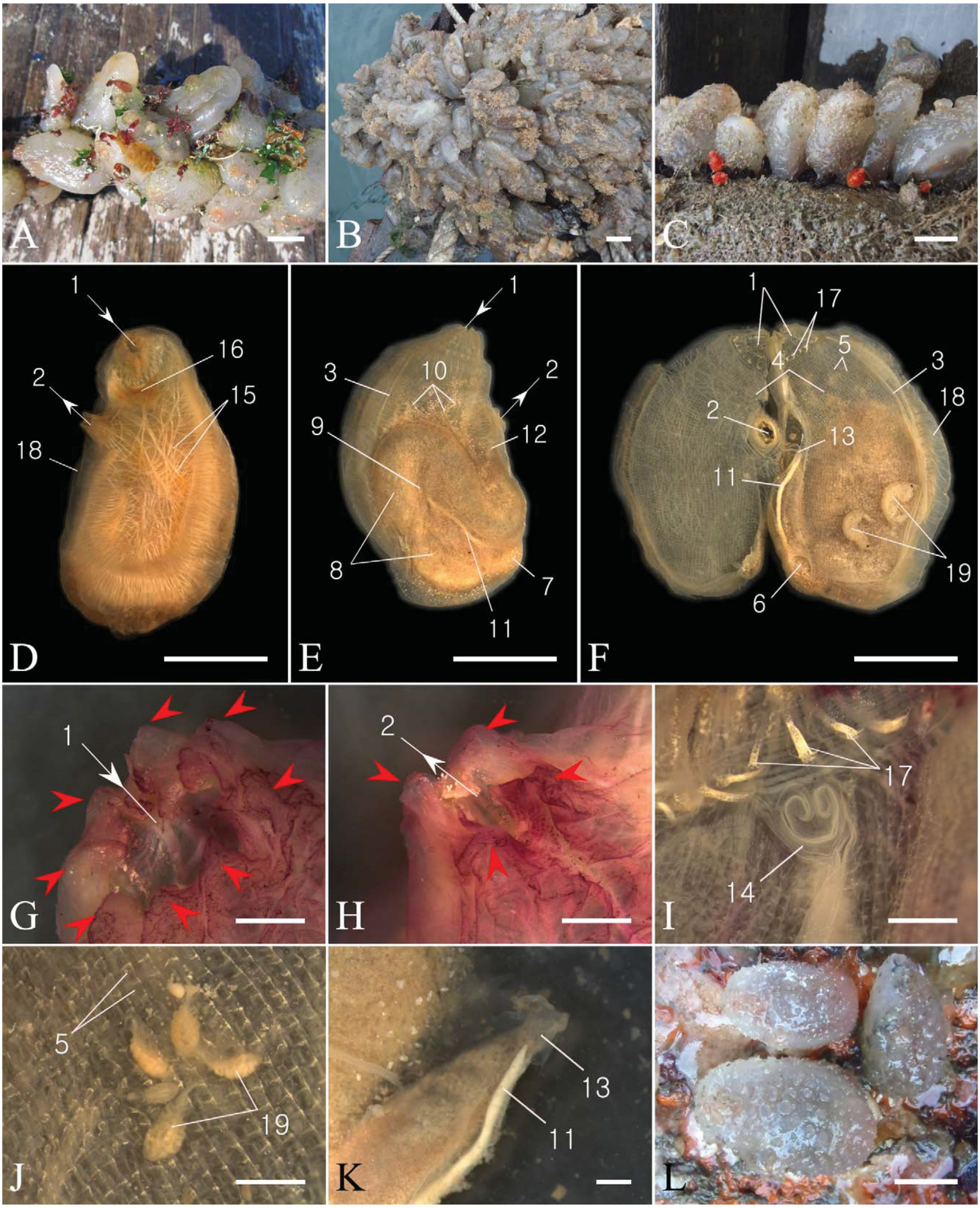

FIGURE 4. *Ascidiella aspersa*: A, B, Individuals densely aggregated on a rope; C, Individuals on the dock wall; D, Right side; E, Left side; F, Sagittal section of branchial sac; G, Oral siphon; H, Atrial siphon; I, Dorsal tubercle; J, Stigmata; K, Anus and gonoduct; L, Individuals. 1—oral siphon, 2—atrial siphon, 3—endostyle; 4—branchial sac, 5—stigmata, 6—esophagus, 7—stomach, 8—intestine, 9—gonads, 10—testis, 11—gonoduct, 12—rectum, 13—anus, 14—dorsal tubercle, 15—body muscles, 16—reddish pigments, 17—tentacles, 18—tunic, 19—parasitic amphipods. Scale bars: A—F, L. 10 mm; G—K. 1 mm.

Size. Individuals measured up to 83 mm long and usually attached on hard substrates by their left side; usual size is 20–47 mm.

Distribution. Native to Norway. Northeast Atlantic (Sweden, Baltic Sea, Irish Sea, English Channel, Germany), Mediterranean Sea, South Africa, New England, Argentina, South Australia, Tasmania, New Zealand, Japan, and Korea (East Sea, Korea Strait, Yellow Sea).

Remarks. This species was widespread in three coastlines of Korea except Jeju Island (Table 3). Some parasitic amphipods were often found in the branchial sac (Figs. 4F, J). *S. plicata* an established alien species in Korea were found attached to a rope and looked like aquacultured organism at Tongyeong yacht marina in October 2010 (Figs. 3A, B). But newly introduced *A. aspersa* occupied the same rope at the same location in June 2011 (Fig. 3C). It has been suggested that *S. plicata* might be replaced by *A. aspersa* in competition for habitats. As shown in Fig. 3, the reduction of individual number of *S. plicata* was considered to be by the loss of food intake due to the attachment of many *A. aspersa* (Fig. 3D). This seasonal change of species needs to be examined continuously.

Family Cionidae Lahille, 1887

Genus *Ciona* Fleming, 1822

3. *Ciona intestinalis* (Linnaeus, 1767)
(Fig. 2C, Table 3)

Ascidia canina Müller, 1776: 43.
Ascidea intestinalis: De Kay, 1843: 259.
Ciona intestinalis: Van Name, 1912: 606, fig. 43, pl. 66, fig. 130; Van Name, 1945: 160, fig. 79; Millar, 1952: 47; Tokioka, 1954: 82; Yamaguchi, 1975: 253; Rho, 1977: 316; Rho & Lee, 1991: 201; Currie *et al*, 1998: 25; Lambert & Lambert, 1998: 675; Stachowicz *et al*., 2002: 15498; Shirley, 2003: 14; Turon & López-Legentil, 2004: 311; Castilla *et al*., 2005: 213; Passamaneck & Gregorio, 2005: 1; Carver *et al*., 2006: 1; Nydam & Harrison, 2007: 1839; Howes *et al*., 2007: 85; Petersen, 2007: 128; Therriault & Herborg, 2008: 788; Picton & Morrow, 2010b: ZD1170; Shenkar *et al*, 2011: 103732; Veeman *et al*., 2011: 401.

Materials examined. 46 inds., Guryoungpo, 12 Aug. 2009, Lee T, thick cloth at 1.2 m depth; 21 inds., Gampo, 12 Aug. 2009, Shin S, thick cloth at 1.3 m depth; 33 inds., Daebyeon, 13 Aug. 2009, Shin S, ropes at 1.1 m depth; 19 inds., Busan, 14 Aug. 2009, Lee T, under floating dock at 0.3 m depth; 39 inds., Dadaepo, 14 Aug. 2009, Lee T, under floating dock at 0.2 m depth; 46 inds., Tongyeong, 14 Aug. 2009, Shin S, ropes at 2.1 m depth; 22 inds., Gwangyang, 16 Aug. 2009, Shin S, fish trap at 3.5 m depth; 37 inds., Guryoungpo, 13 Nov. 2009, Lee T, thick cloth at 1.2 m depth; 18 inds., Gampo, 13 Nov. 2009, Lee T, under floating dock at 0.3 m depth; 29 inds., Daebyeon, 14 Nov. 2009, Shin S, ropes at 3.5 m depth; 13 inds., Busan, 14 Nov. 2009, Shin S,, ropes at 3.1 m depth; 25 inds., Dadaepo, 14 Nov. 2009, Lee T, under floating dock at 0.2 m depth; 46 inds., Tongyeong, 15 Nov. 2009, Shin S, dock wall at 1.7 m depth; 16 inds., Gwangyang, 15 Nov. 2009, Lee T, fish trap at 3.2 m depth; 46 inds., Guryoungpo, 24 June 2010, Lee T, ropes at 3 m depth; 7 inds., Yangpo, 24 June 2010, Shin S, under floating dock at 0.3 m depth; 22 inds., Gampo, 24 June 2010, Shin S, thick cloth at 1.3 m depth; 26 inds., Bangeojin, 25 June 2010, Lee T, ropes at 1.1 m depth; 6 inds., Jangsaengpo, 25 June 2010, Lee T, ropes at 1.1 m depth; 35 inds., Daebyeon, 25 June 2010, Shin S, fish trap at 3.5 m depth; 13 inds., Busan, 25 June 2010, Lee T, under floating dock at 0.3 m depth; 29 inds., Dadaepo, 26 June 2010, Lee T, under floating dock at 0.2 m depth; 79 inds., Tongyeong, 26 June 2010, Shin S, ropes at 2.1 m depth; 22 inds., Gwangyang, 27 June 2010, Lee T, fish trap at 3.5 m depth; 13 inds., Mokpo, 28 June 2010, Shin S, ropes at 1.1 m depth; 9 inds., Gunsan, 29 June 2010, Shin S, under floating dock at 0.3 m depth; 2 inds., Daejin, 17 Oct. 2010, Shin S, ropes at 2.7 m depth; 5 inds., Ayajin, 17 Oct. 2010, Pyo J, fish trap at 3.7 m depth; 6 inds., Sacheon, 18 Oct. 2010, Shin S, thick cloth at 1.9 m depth; 27 inds., Guryoungpo, 19 Oct. 2010, Shin S, ropes at 2.7 m depth; 26 inds., Yangpo, 19 Oct. 2010, Shin S, ropes at 2.7 m depth; 18 inds., Gampo, 19 Oct. 2010, Shin S, fish trap at 2.1 m depth; 9 inds., Bangeojin, 20 Oct. 2010, Shin S, thick cloth at 1.2 m depth; 6 inds., Jangsaengpo, 20 Oct. 2010, Lee T, dock wall at 2 m depth; 17 inds., Daebyeon, 20 Oct. 2010, Pyo J, fish trap at 2.7 m depth; 4 inds., Busan, 20 Oct. 2010, Pyo J, ropes at 2.1 m depth; 23 inds., Dadaepo, 21 Oct. 2010, Pyo J, dock wall at 3 m depth; 41 inds., Tongyeong, 21 Oct. 2010, Pyo J, fish trap at 3.3 m depth; 4 inds.,

Daejin, 22 June 2011, Lee T, ropes at 2.3 m depth; 36 inds., Guryoungpo, 24 June 2011, Shin S, ropes at 2.1 m depth; 25 inds., Yangpo, 24 June 2011, Pyo J, ropes at 2.6 m depth; 59 inds., Gampo, 24 June 2011, Shin S, thick cloth at 1.5 m depth; 7 inds., Bangeojin, 25 June 2011, Lee T, ropes at 2.7 m depth; 32 inds., Daebyeon, 25 June 2011, Pyo J, dock wall at 3 m depth; 5 inds., Busan, 25 June 2011, Lee T, ropes at 2.3 m depth; 18 inds., Dadaepo, 25 June 2011, Pyo J, under floating dock at 0.2 m depth; 331 inds., Tongyeong, 26 June 2011, Pyo J, ropes at 3.3 m depth; 14 inds., Gwangyang, 26 June 2011, Shin S, fish trap at 3.3 m depth; 2 inds., Daejin, 16 Oct. 2011, Shin S, ropes at 1.9 m depth; 5 inds., Geojin, 16 Oct. 2011, Pyo J, ropes at 1.8 m depth; 7 inds., Jumunjin, 17 Oct. 2011, Lee T, under floating dock at 0.1 m depth; 4 inds., Sacheon, 17 Oct. 2011, Pyo J, ropes at 2 m depth; 2 inds., Gangneung, 17 Oct. 2011, Shin S, thick cloth at 2 m depth; 1 inds., Ganggu, 18 Oct. 2011, Lee T, fish trap at 3.2 m depth; 58 inds., Guryoungpo, 18 Oct. 2011, Lee T, dock wall at 3 m depth; 27 inds., Yangpo, 18 Oct. 2011, Shin S, ropes at 2.1 m depth; 86 inds., Gampo, 18 Oct. 2011, Lee T, thick cloth at 1.5 m depth; 14 inds., Bangeojin, 19 Oct. 2011, Pyo J, ropes at 2.7 m depth; 4 inds., Jangsaengpo, 19 Oct. 2011, Shin S, fish trap at 3 m depth; 13 inds., Daebyeon, 19 Oct. 2011, Lee T, ropes at 2.8 m depth; 8 inds., Songjeong, 19 Oct. 2011, Pyo J, under floating dock at 0.2 m depth; 3 inds., Busan, 19 Oct. 2011, Pyo J, dock wall at 1.6 m depth; 25 inds., Dadaepo, 19 Oct. 2011, Lee T, fish trap at 3.2 m depth; 64 inds., Tongyeong, 20 Oct. 2011, Shin S, dock wall at 2.1 m depth; 12 inds., Gwangyang, 20 Oct. 2011, Pyo J, fish trap at 3.2 m depth.

Distribution. Native all around the British Isles and reported in many part of the world. Northern Europe, part of Arctic regions, Mediterranean Sea, Black Sea, Denmark, France, Atlantic Canada, New England, South Africa, Washington, California, Chile, South Australia, Tasmania, New Zealand, China, Japan, and Korea (East Sea, Korea Strait, Jeju Island, Yellow Sea).

Order Stolidobranchia Lahille, 1887

Family Molgulidae Lacaze-Duthiers, 1877

Genus *Molgula* Forbes, 1848

4. *Molgula manhattensis* (De Kay, 1843)

(Figs. 2D, 5, Table 3)

Ascidea manhattensis De Kay, 1843: 259.

Caesira manhattensis: Van Name, 1912: 471, figs. 4, 5, pl. 45, figs. 11-15, pl.71, figs. 151, 152

Molgula manhattensis: Berrill, 1928: 163; Van Name, 1945: 385, figs. 271-273; Tokioka & Kado, 1972: 21; Lambert & Lambert, 1998: 675; Stachowicz *et al*., 2002: 2576; Carman *et al*., 2007: 175; Hiscock, 2008: 3823; Carman & Grunden, 2010: 23; Haydar *et al*., 2011: 68; Sanamyan & Monniot, 2011c: 103788.

Materials examined. 35 inds., Mokpo, 26 June 2010, Lee T, fish trap at 2.5 m depth; 47 inds., Gunsan, 27 June 2010, Shin S, under floating dock at 0.2 m depth; 84 inds., Mokpo, 21 Oct. 2010, Pyo J, fish trap at 2.1 m depth; 26 inds., Gunsan, 22 Oct. 2010, Shin S, under floating dock at 0.1 m depth; 41 inds., Incheon, 22 Oct. 2010, Lee T, ropes at 3.2 m depth; 12 inds., Gwangyang, 26 June 2011, Lee T, fish trap at 3.4 m depth; 56 inds., Mokpo, 26 June 2011, Lee T, under floating dock at 0.2 m depth; 33 inds., Incheon, 27 June 2011, Pyo J, ropes at 2.7 m depth; 6 inds., Songjeong, 19 Oct. 2011, Pyo J, dock wall at 1.1 m depth; 7 inds., Busan, 19 Oct. 2011, Pyo J, under floating dock at 0.2 m depth; 12 inds., Dadaepo, 19 Oct. 2011, Shin S, ropes at 2.2 m depth; 35 inds., Mokpo, 20 Oct. 2011, Lee T, under floating dock at 0.1 m depth; 21 inds., Incheon, 21 Oct. 2011, Shin S, ropes at 3.8 m depth.

Description. Body commonly ovoid shaped (Fig. 5A–C, L). Tunic thick, grayish or greenish color, semi-transparent, sometimes covered with debris and fibrils; visceral organs observed by naked eye on each side. Oral siphon 6 lobed, positioned at anterior part of body (Fig. 5G). Six to eight tentacles surround opening of oral siphon into pharynx (Fig. 5H), and each tentacle irregularly branched 7–16 ramification. Atrial siphon 4 lobed, positioned closed beside oral siphon less than 5 mm (Fig. 5G). Dorsal tubercle C-shaped or horseshoe-shaped groove (Fig. 5I). Branchial sac with six folds, on each side curved and spirally arranged stigmata (Figs. 5J, K). Muscles well developed around siphons. Endostyle extended from base of oral siphon to posterior of pharynx. Gut positioned on left side of branchial sac (Fig. 5E). Stomach narrow, small, completely hidden by large conspicuous pyloric gland,

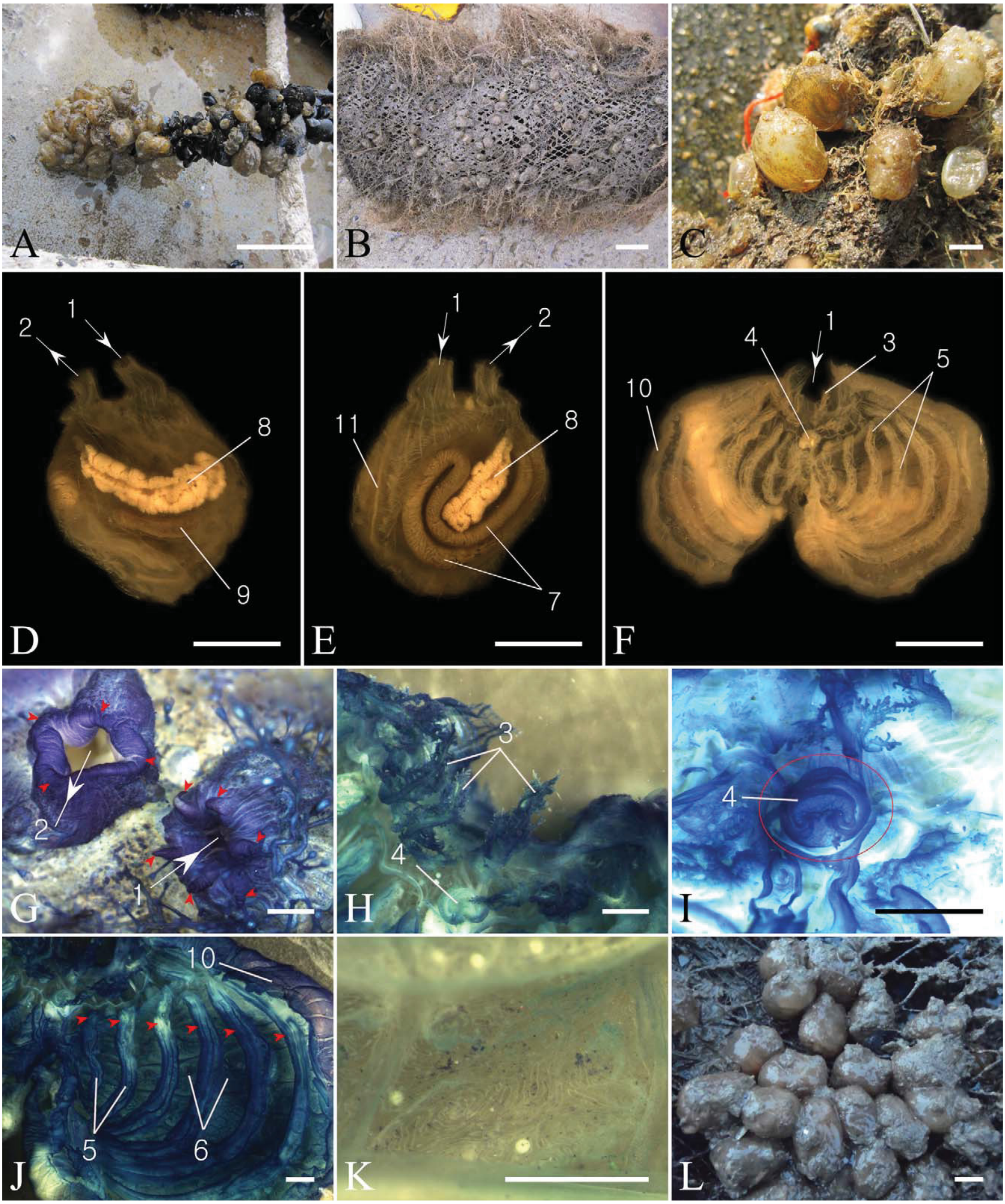

FIGURE 5. *Molgula manhattensis*: A, C, Individuals densely aggregated on a rope; B, Individuals on a fish trap; D, Right side; E, Left side; F, Sagittal section of branchial sac; G, Oral and Atrial siphon; H, Tentacles; I, Dorsal tubercle; J, Six folds of branchial sac; K, Stigmata; L, Individuals attached to fish trap. 1—oral siphon, 2—atrial siphon, 3—tentacles, 4—dorsal tubercle, 5—folds of branchial sac, 6—stigmata, 7—intestine, 8—gonads, 9—renal sac, 10—tunic, 11—endostyle. Scale bars: A–B. 50 mm; C–F, L. 5 mm; G–K. 1 mm.

but not exactly marked off from rest of gut. Intestine tapering from end of stomach, gonad in second gut loop. Renal sac positioned beneath gonads on body wall of right side, slightly curved (Fig. 5D). Gonads located on each side, consist of testis and ovary surrounded by testis; right gonad deflected, positioned parallel with renal sac and at right angle with left gonad (Figs. 5D, E). Oviduct short, extends toward atrial siphon.

Size. Individuals measured up to 24 mm long and usually attached to ropes and hard substrates by posterior part; usual size is 15–20 mm.

Distribution. Native to Northeast America from Maine to Texas. Northeast Atlantic (Norway to Portugal), Northeast Pacific (Puget Sound, San Juan Archipelago, Vancouver Island, California), South Australia, Tasmania, China, Japan, and Korea (Korea Strait, Yellow Sea).

Remarks. *Molgula manhattensis* (De Kay, 1843) was first described from New York harbor. Its distribution in Northwest Atlantic extends from Cape Cod to Southern Louisiana interrupted by the Florida peninsula (Van Name 1945). Although its European distribution extends from Norway to Portugal, it is patchy (Monniot 1969). The inferred anthropogenic vectors for these introductions were hull fouling and oyster translocations (Tokioka & Kado 1972; Cohen & Carlton 1996), and possibly ballast water (Hewitt *et al*. 2004). This species was first found in Mokpo, Gunsan and Incheon of Yellow Sea, Korea in June 2010 and was found spread out at Songjeong, Busan and Dadaepo of Korea Strait in 2011 (Table 3).

Family Styelidae Sluiter, 1895

Genus *Styela* Fleming, 1822

5. *Styela plicata* (Lesueur, 1823)

(Figs. 2E, 3A, B, Table 3)

Ascidia plicata Lesueur, 1823: 5; De Kay, 1843: 259.
Styela pinguis Herdman, 1899: 37.
Styela plicata: Herdman, 1899: 40; Van Name, 1945: 295, figs. 192, 194; Tokioka, 1954: 89; 1960: 212; Kott, 1972: 185; Yamaguchi, 1975: 253; Rho & Lee, 1991: 202; Lambert & Lambert, 1998: 675; Rho *et al*., 2000: 42; Baker *et al*., 2004: 74; Da Rocha & Kremer, 2005: 1170; De Barros *et al*., 2009: 45; Pineda *et al*., 2011: 1; Sanamyan & Monniot, 2011d: 103936; Fuller, 2012: 1293.
Tethyum plicatum: Van Name, 1912: 569, fig. 136.

Materials examined. 8 inds., Guryoungpo, 12 Aug. 2009, Lee T, thick cloth at 1.4 m depth; 14 inds., Gampo, 12 Aug. 2009, Shin S, thick cloth at 2.1 m depth; 7 inds., Jangsaengpo, 13 Aug. 2009, Shin S, ropes at 2.4 m depth; 16 inds., Daebyeon, 13 Aug. 2009, Shin S, ropes at 3.1 m depth; 8 inds., Busan, 14 Aug. 2009, Lee T, under floating dock at 0.3 m depth; 3 inds., Dadaepo, 14 Aug. 2009, Lee T, under floating dock at 0.2 m depth; 269 inds., Tongyeong, 14 Aug. 2009, Shin S, ropes at 2.5 m depth; 11 inds., Gwangyang, 16 Aug. 2009, Shin S, fish trap at 3.7 m depth; 6 inds., Seogwipo, 20 Aug. 2009, Shin S, ropes at 2.5 m depth; 3 inds., Guryoungpo, 13 Nov. 2009, Lee T, thick cloth at 1 m depth; 6 inds., Gampo, 13 Nov. 2009, Lee T, under floating dock at 0.2 m depth; 6 inds., Jangsaengpo, 14 Nov. 2009, Shin S, ropes at 2.5 m depth; 5 inds., Daebyeon, 14 Nov. 2009, Shin S, ropes at 3.8 m depth; 10 inds., Busan, 14 Nov. 2009, Shin S,, ropes at 3.1 m depth; 9 inds., Dadaepo, 14 Nov. 2009, Lee T, under floating dock at 0.2 m depth; 290 inds., Tongyeong, 15 Nov. 2009, Shin S, dock wall at 1.7 m depth; 14 inds., Gwangyang, 15 Nov. 2009, Lee T, fish trap at 3.1 m depth; 8 inds., Seogwipo, 17 Nov. 2009, Shin S, ropes at 3.1 m depth; 13 inds., Yangpo, 24 June 2010, Shin S, ropes at 3 m depth; 16 inds., Bangeojin, 25 June 2010, Shin S, ropes at 2.1 m depth; 5 inds., Jangsaengpo, 25 June 2010, Lee T, fish trap at 3.7 m depth; 4 inds., Busan, 25 June 2010, Lee T, under floating dock at 0.1 m depth; 16 inds., Dadaepo, 26 June 2010, Shin S, ropes at 2.6 m depth; 327 inds., Tongyeong, 26 June 2010, Shin S, dock wall at 2.8 m depth; 9 inds., Gwangyang, 27 June 2010, Lee T, fish trap at 3.2 m depth; 10 inds., Seogwipo, 30 June. 2010, Lee T, ropes at 3.4 m depth; 31 inds., Yangpo, 19 Oct. 2010, Shin S, ropes at 3.2 m depth; 19 inds., Gampo, 19 Oct. 2010, Pyo J, ropes at 2.7 m depth; 4 inds., Jangsaengpo, 20 Oct. 2010, Lee T, dock wall at 2 m depth; 8 inds., Busan, 20 Oct. 2010, Pyo J, dock wall at 3 m depth; 24 inds., Dadaepo, 21 Oct. 2010, Shin S, ropes at 2.1 m depth; 547 inds., Tongyeong, 21 Oct. 2010, Shin S, fish trap at 3.3 m depth; 11 inds., Gwangyang, 21 Oct. 2010, Lee T, fish trap at 2.5 m depth; 20 inds., Seogwipo, 17 Oct. 2010, Pyo J, ropes at 2.1 m depth; 21 inds., Yangpo, 24 June 2011, Shin S, ropes at 3.4 m depth; 17 inds., Bangeojin, 25 June

2011, Shin S, ropes at 2.2 m depth; 9 inds., Jangsaengpo, 25 June 2011, Lee T, fish trap at 2.3 m depth; 5 inds., Busan, 25 June 2011, Pyo J, under floating dock at 0.2 m depth; 12 inds., Dadaepo, 25 June 2011, Shin S, ropes at 2.6 m depth; 75 inds., Tongyeong, 26 June 2011, Pyo J, dock wall at 3.8 m depth; 9 inds., Seogwipo, 17 June 2011, Pyo J, ropes at 3.4 m depth; 20 inds., Yangpo, 18 Oct. 2011, Shin S, ropes at 2.1 m depth; 11 inds., Jangsaengpo, 19 Oct. 2011, Lee T, fish trap at 3.1 m depth; 9 inds., Busan, 19 Oct. 2011, Pyo J, dock wall at 1.6 m depth; 16 inds., Dadaepo, 19 Oct. 2011, Lee T, under floating dock at 0.2 m depth; 323 inds., Tongyeong, 20 Oct. 2011, Shin S, ropes at 3.1 m depth; 6 inds., Gwangyang, 20 Oct. 2011, Pyo J, fish trap at 3.2 m depth; 13 inds., Seogwipo, 25 Oct. 2011, Pyo J, ropes at 3 m depth.

Distribution. Native to Northeast America, Gulf of Mexico and West Indies. Mediterranean Sea, South Africa, Brazil, Argentina, California, South Australia, Tasmania, China, Japan, and Korea (Korea Strait, Jeju Island).

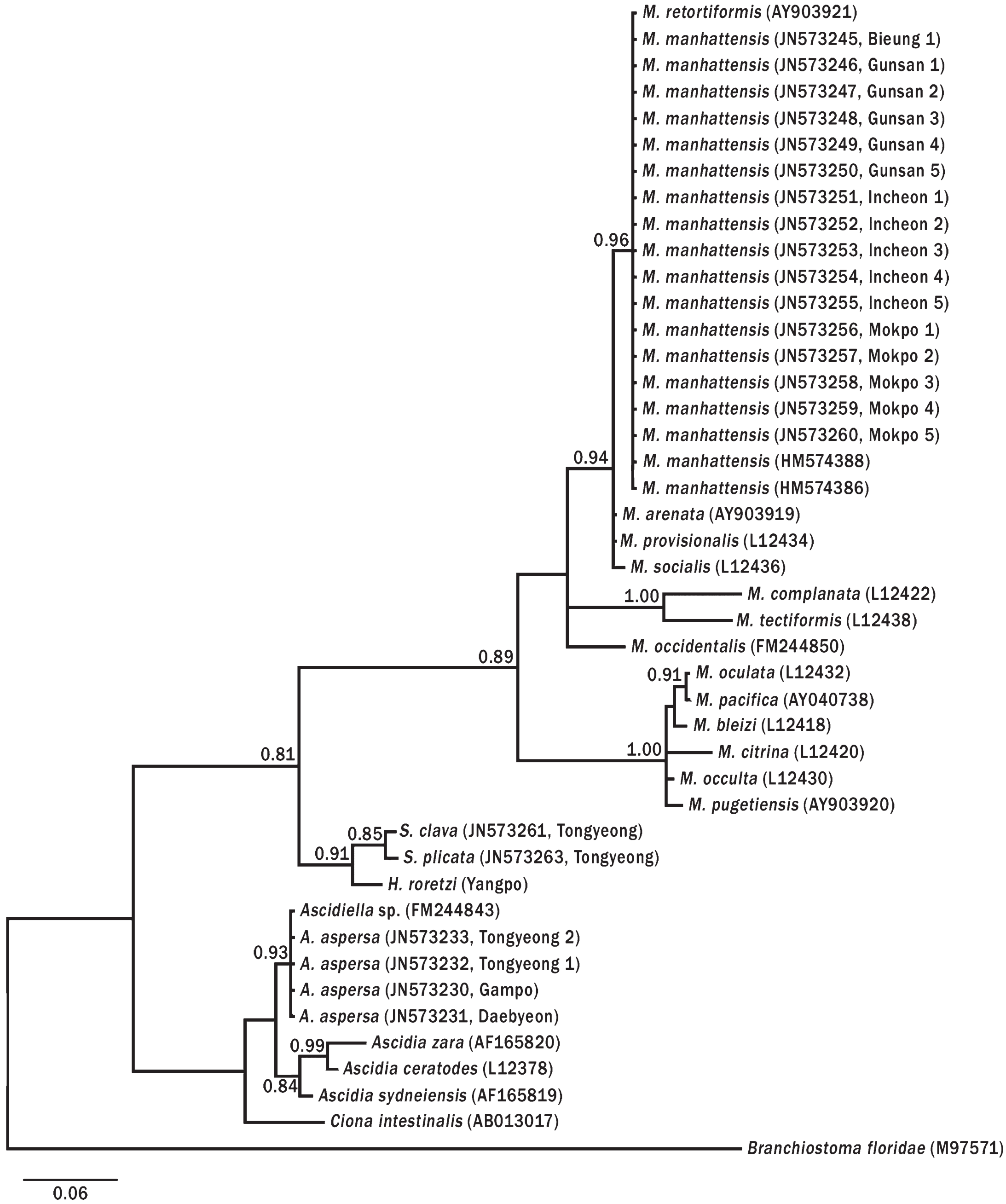

FIGURE 6. Phylogenetic relationship of some ascidians including four invasive alien species inferred from nuclear 18S rDNA dataset. Species, sampling locations in Korea and Genbank accession numbers were given and numbers on branches were Bayesian posterior probabilities (if ≥ 0.80). The tree was rooted with *Branchiostoma floridae*. The scale bar represents the number of expected changes per site.

Molecular phylogenetic analysis. In the jModeltest procedure, the Akaike Information Criterion (AIC) showed that the GTR+I+G model was the best-fitted model among those evaluated. The parameters of the model were as follows: base frequencies, $A = 0.2383$, $C = 0.2272$, $G = 0.3050$, $T = 0.2295$; substitution rate matrix, $A–C = 0.3903$, $A–G = 1.4160$, $A–T = 0.8681$, $C–G = 0.6066$, $C–T = 4.0151$, $G–T = 1.0000$; proportion of invariable sites 0.4250; gamma shape parameter 0.4350. We investigated the phylogenetic relationships between five Korean ascidian species and the allied species registered in the GenBank. The analysis of the base sequences of Korean ascidians was carried out using twenty-three samples of nuclear 18S rDNA and ten of mt-COI. Korean ascidians used for the analysis of 18S rDNA sequences were *Ascidiella aspersa, Halocynthia roretzi, Molgula manhattensis, Styela clava,* and *S. plicata,* among which *A. aspersa*, *M. manhattensis* and *S. plicata* were also used for mt-COI analysis. These base sequences were employed for the construction of the phylogenetic tree by Bayesian inference method and the trees obtained using each sequence of nuclear 18S rDNA (Fig. 6) and mt-COI (Fig. 7). Korean *A. aspersa* exactly formed a phylogenetic group with same species referred from GenBank in each phylogenetic tree of nuclear 18S rDNA and mt-COI (Figs. 6, 7), and made particularly significant distinction from the other species of genus *Ascidia* of the same family in phylogenetic tree of nuclear 18S rDNA (Fig. 6). Korean *M. manhattensis* also formed a phylogenetic group with same species from Genbank in both phylogenetic trees of nuclear 18S rDNA and mt-COI. But, *M. manhattensis* was grouped with *M. retortiformis* in nuclear 18S rDNA dataset (Fig. 6) and with *M. provisionalis* in mt-COI dataset (Fig. 7).

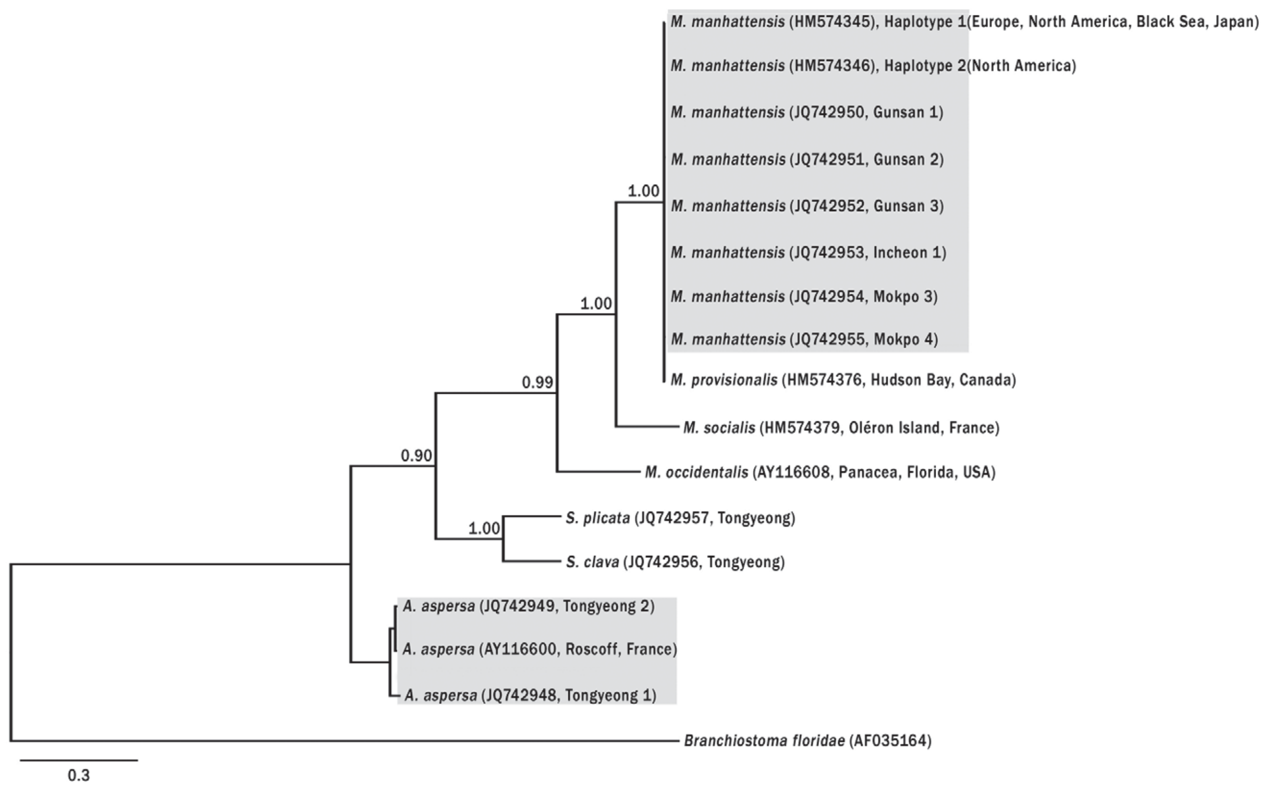

FIGURE 7. Phylogenetic relationship of some ascidians including three invasive alien species inferred from mt–COI dataset. Species, sampling locations in Korea and Genbank accession numbers were given and numbers on branches were Bayesian posterior probabilities (if ≥ 0.80). The tree was rooted with *Branchiostoma floridae*. The scale bar represents the number of expected changes per site.

Discussion

The present study was designed to examine the phylogenetic relationship of Korean ascidians using nuclear 18S rDNA and mt-COI. Molecular results analyzed for the ascidian identified in this study conformed to the results of nuclear 18S rDNA (Hadfield *et al*. 1995; Huber *et al*. 2000; Zeng *et al*. 2006) and mt-COI (Haydar *et al*. 2011). Korean *A. aspersa*

distinctly forms a monophyletic group with same species referred from GenBank in the phylogenetic trees of nuclear 18S rDNA (Fig. 6) and mt-COI (Fig. 7), and these results supported the validity of morphological identification. All *Molgula* species formed the monophyletic group that has been highly supported (Zeng *et al.* 2006). Several sequences of *M. manhattensis* collected from different collection sites distinctly formed a group in both phylogeny trees of nuclear 18S rDNA and mt-COI. This species was grouped with *M. retortiformis* which was morphologically different from *M. manhattensis* (Fig. 6), but according to the analysis of mt-COI sequences, this species was grouped with *M. provionalis* which was similar to *M. manhattensis* (Fig. 7). These results were coincident with the reports of Zeng *et al.* (2006) and Haydar *et al.* (2011). In the morphological study of Van Name (1945), *M. retortiformis* was usually 30–40 mm in diameter, and reached the maximum size of 100 mm in the Bering Sea; the numbers of folding of branchial sac were seven; the first gut loop was located horizontally; and the ovary and the testis were completely separated from each other; the ovary positioned in the second gut loop and the testis in the first gut loop; the ovary positioned right above the renal sac and the testis right below the renal sac. In case of *M. manhattensis*, the numbers of folding of branchial sac were six; the ovary and testis were combined and located in both sides of the body and positioned in the second gut loop and right above the renal sac. *M. manhattensis* was shown to be very similar to *M. provisionalis* in comparison of external and internal features (Table 4). As described above, the distinct morphological differences were presented between *M. manhattensis* and *M. retortiformis*, but the results of nuclear 18S rDNA analysis demonstrated that both species belonged to the same phylogenetic group (Fig. 6). The results of mt-COI analysis showed that *M. manhattensis* and *M. provisionalis* also belonged to the same phylogenetic group (Fig. 7). This suggests that mt-COI is more useful than nuclear 18S rDNA, and that the taxonomical relationship between *M. manhattensis* and *M. provisionalis* needs to be reexamined through the rigorous morphological comparisons.

TABLE 4. Morphological comparisons between three *Molgula* species.

Characters	*M. retortiformis*	*M. manhattensis*	*M. provisionalis*
Size	about 30–40 mm	about 20 mm	about 10 mm
Folds of branchial sac	7	6	6
Location of ovary and testis	separated; ovary above renal sac testis beneath renal sac	combined; both above renal sac	combined; both above renal sac
Population status	aggregated	aggregated	not aggregated

Description of *M. retortiformis* and *M. provisionalis* as from Van Name (1945).

Acknowledgements

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